

Reproduction of *Stylochus ellipticus* (Platyhelminthes: Polycladida) in Response to Temperature, Food, and Presence or Absence of a Partner

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Abstract. The common polyclad turbellarian *Stylochus ellipticus* is an important and abundant predator of young oysters, *Crassostrea virginica*, in Chesapeake Bay. Laboratory experiments revealed that higher temperature and starvation negatively affected flatworm size. Egg production, adjusted for flatworm area, was not significantly affected by flatworm size, nor by increasing number of egg batches produced. In addition, there was no significant statistical effect of higher temperature and starvation on egg production, although there were some instances of diminished egg production under these conditions. Flatworms did react to higher temperature and starvation by significantly decreasing the number of days that elapsed before egg laying began. Overall, more larvae would be produced at 21°C than at 30°C, by fed flatworms than starved flatworms, and by solitary flatworms than paired flatworms. When isolated animals were alternately fed and starved for five-week periods over a 20-week experimental period at 21°C, 56% of individuals continued to deposit eggs, although the number of eggs laid and embryo hatching success declined with time. *Stylochus ellipticus* individuals can maintain a relatively high reproductive effort despite reduction in available food. Thus, this species would appear to be a "capital" breeder, relying on stored energy to produce eggs.

Introduction

The free-living flatworm *Stylochus ellipticus* (Girard) is usually found in littoral and sublittoral habitats (Hyman, 1940, 1951; Loosanoff, 1956) and is common on

U.S. Atlantic and Gulf of Mexico coasts. It occurs at low to moderate salinities (Pearse and Wharton, 1938; Landers and Rhodes, 1970) over a wide temperature range. This flatworm is an active predator of eastern oyster spat [*Crassostrea virginica* (Gmelin)] and barnacles of various species in Chesapeake Bay and its tributaries (Webster and Medford, 1961; Shaw, 1967; Christensen, 1973). Natural oyster mortality has been associated with flatworm density in Chesapeake Bay (Webster and Medford, 1961). Ralfi-cultured oyster spat have been heavily preyed upon by the flatworm in Chesapeake Bay (Christensen, 1973) and in Massachusetts (Provenzano, 1961).

The reproductive pattern and life history of *S. ellipticus* are poorly understood. For example, *S. ellipticus* is hermaphroditic, but is generally thought to be cross-fertilizing (Hyman, 1940, 1951; Prudhoe, 1985) because the male and female copulatory organs are separate (Prudhoe, 1985). However, because the male and female genital pores are close together, self-fertilization (which occurs among other groups of flatworms) may be possible (Prudhoe, 1985).

While holding individuals of *S. ellipticus* separately in petri dishes early in this study, we noticed that they kept laying eggs. There appeared to be no information in the literature on the need for continued contact with other flatworms to maintain production of eggs, fertilized or not. In addition, there was a lack of information on the effects of environmental factors on reproduction in this species. Consequently, we undertook exploratory laboratory studies to examine how late spring and late summer temperatures, being fed or starved, and the presence or absence of a potential sexual partner ("status") affected reproduction in *S. ellipticus*. Evidence presented here reveals that individuals are fecund even in the face of high

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temperature, starvation, and the lack of a partner, with about 93–100% (median values) of the eggs produced eventually hatching.

Materials and Methods

General procedures

Flatworms were collected in the spring and summer by dredging oysters and oyster shells from oyster bars in the Choptank River (a tributary of central Chesapeake Bay). Salinities in the river ranged between 10 and 16 ppt during the study. Flatworms were held singly in the dark in 85-mm petri dishes at ambient salinity and room temperature for 24–48 h after collection. Apparently healthy individuals were then measured and used in the experiments (again, in 85-mm petri dishes) described below. In the paired experiments, flatworms were placed with worms of the same relative size. The flatworms were then assigned haphazardly to the respective treatments.

To avoid injury to specimens, we measured the size (area) of each flatworm, with as little disturbance as possible, by observing it through a dissection microscope while it crawled in a petri dish placed on graph paper (Lytwyn and McDermott, 1976). Low-intensity light induced movement away from the light. Length (anterior-posterior axis) and width (perpendicular to the anterior-posterior axis) were measured in terms of the number of graph squares covered while the animal was fully extended, with the number of graph squares subsequently converted to mm^2 . Because these animals are elastic when moving, a surface area estimate of length times width was thought to be preferable to a length measurement alone. The replicability of this area measurement was determined at the start of the study by measuring each of seven animals twice, with a resultant 4% deviation from the mean. We measured adult flatworms weekly to monitor growth or shrinkage ("degrowth"; Calow and Woollhead, 1977) during the experiments; size changes are presented as the area when a treatment or an experiment ended expressed as a percentage of the area at the start.

During experiments, we checked the petri dishes every 48 h for the presence of eggs and changed the water (and food if flatworms had been fed). Because it is difficult to control the amount of food offered in experiments on small aquatic carnivores, we chose to offer one consistent amount of food to some flatworms and none to others in these exploratory experiments. Thus, flatworms that were fed were given a piece of fresh oyster body tissue (one piece for solitary flatworms; two for pairs; gills were not included in the tissue) that was about 4 mm^2 in size. In many instances, the food was consumed within 48 h. Uneaten food was discarded and replaced with fresh tissue (we have no data on possible declines in oxygen tension as uneaten food decayed). If egg masses were present, the

flatworm(s) was (were) moved to a new dish. We designate the number of eggs laid by a flatworm or pair of flatworms during a 48-h period as a "batch," and note that the disturbances associated with changing water and dishes may have interrupted egg-laying episodes.

The effects of experimental manipulations were assessed by determining change in flatworm area, as well as the following estimators of reproductive effort: number of flatworms that laid eggs, number of days to the laying of the first batch, number of batches per flatworm or flatworm pair, number of eggs per batch, and percentage of embryos hatching in each batch. All mean values are reported ± 1 standard error of the mean except when percentages are involved; in those instances the median and range are reported. On occasion, flatworms crawled out of the water onto the covers of the petri dishes and desiccated, and a few were found dead of unknown causes in the water. Data from these animals were not used, but the mortalities are reported.

When eggs were present, they were teased off the petri dish with a paint brush and preserved with formalin for later enumeration. A subsample of 50–100 eggs was allowed to remain in each dish so we could monitor hatching success. We counted the number of eggs in the subsample by microscopic examination, and returned the subsample to its rearing regime of darkness, salinity, and temperature until hatching occurred. Water was changed every 2 days. Embryos that showed no signs of development after 7 days (embryos usually hatched 5–7 days after eggs were laid), nor the rotating motion characteristic of pre-hatch behavior, were considered to be nonviable, allowing us to determine hatching success of that subsample, and by extension, of the batch from which it was derived.

All embryos in the preserved sample were counted, or, if numbers were high, were estimated by subsampling. At the start of the experiments, the accuracy of subsample estimates was checked by first counting the number of embryos in a subsample on a Sedgewick-Rafter slide and multiplying the count by the volume (estimated in a graduated cylinder) of the entire sample, and then by counting the entire sample. A 7% deviation from the mean resulted, so we used this subsampling procedure throughout our studies. Average egg number in all experiments is reported to the nearest hundred eggs, with standard errors rounded to the nearest ten.

The size range of experimental flatworms was high (5–338 mm^2), so in some instances we attempted to adjust for this variation by dividing the number of eggs produced during the experiments by the surface area of solitary worms at the start of an experiment (we call this the "initial size") to provide an area-specific estimate of egg production. This was not done for paired worms because we did not know which flatworm of a pair had laid the eggs.

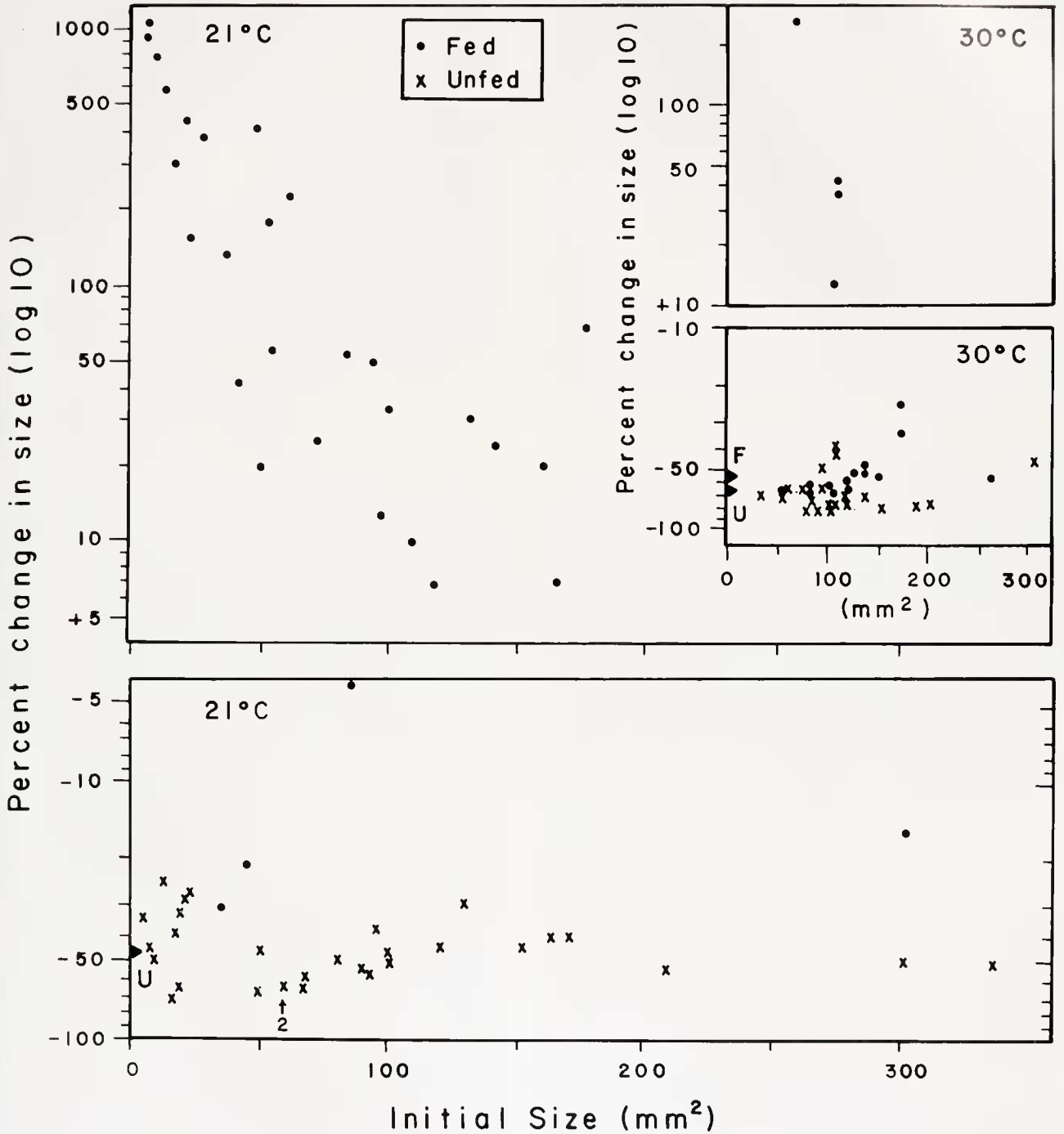
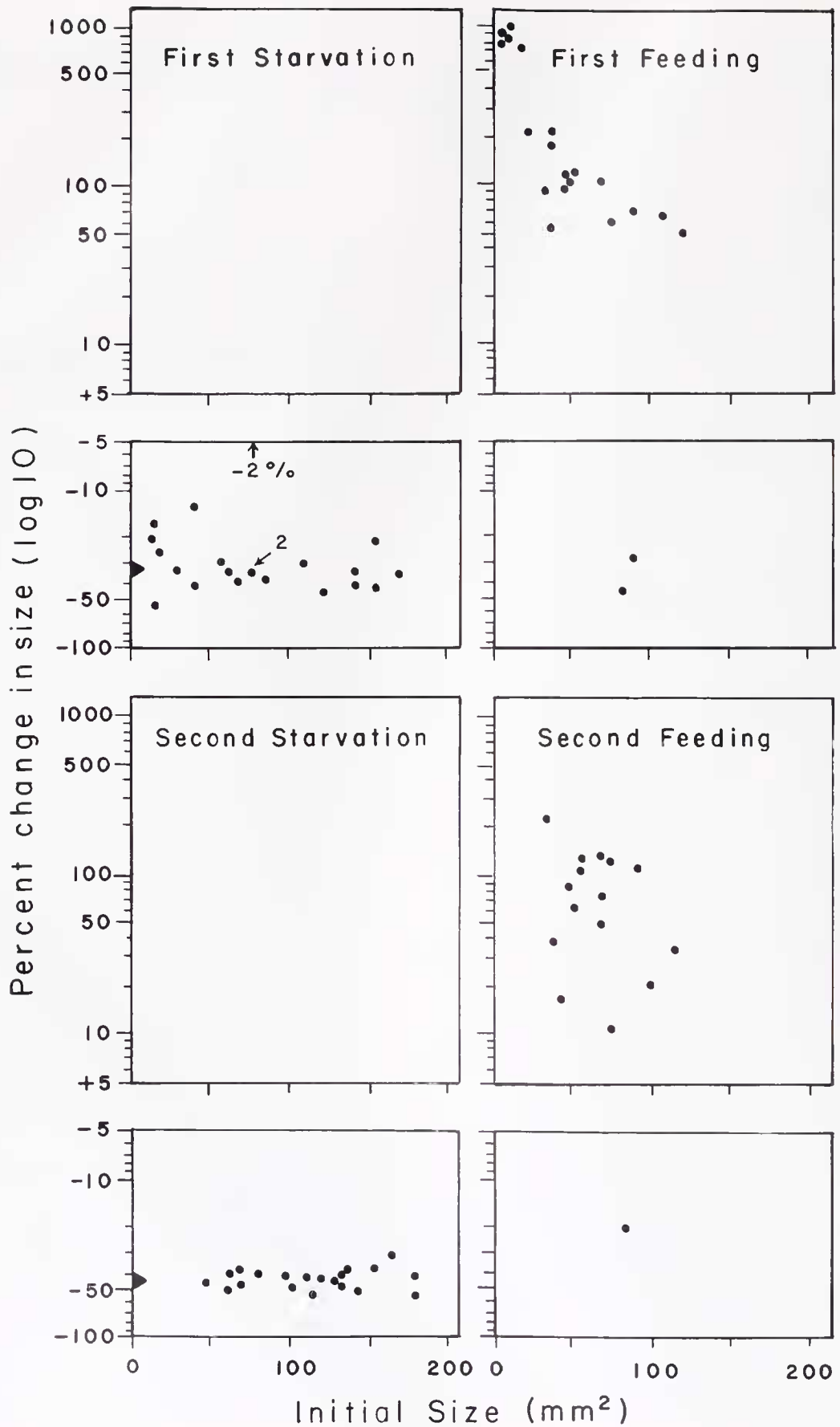


Figure 1. Experiment 1. Percentage increase (+) or decrease (-) in size (length \times width) of solitary *Stylochus ellipticus* in relation to temperature (21°, 30°C) and food (fed, unfed) for 5-week periods. Note that the scale breaks across the main figure (21°C data) and inset figure (30°C data) indicate transition between growth (+) and degrowth (-). Arrowheads on the y-axes indicate average decrease in size for unfed (U) animals at 21°C and for fed (F) and unfed (U) animals at 30°C (inset). Numeral (2) in lower panel at 21°C indicates where data for two animals coincide.



Data were analyzed by a split-level ANOVA (Experiment A, below) because the experiments were run at different times of the year and by regular ANOVA (Experiment B, below) on SAS (Hendrix *et al.*, 1982). An F_{\max} test was used to determine homogeneity of variances, and normality of the data was determined by stem leaf diagrams of the residuals and normal probability plots on SAS. Any data that did not meet these assumptions were transformed prior to analysis. Statistical comparisons were declared to be significant at the $P < 0.1$ level in Experiment A because the high variability among the data and the limits of sample sizes in some instances undoubtedly resulted in a low power of the test (Peterman, 1990). We used a significance level of $P < 0.05$ in Experiment B.

Experiment A: Effects of temperature, food, and presence or absence of a partner

In 1987, flatworm reproduction was assessed over arbitrarily selected 5-week periods in the dark under two feeding regimes (fed, unfed), at two temperatures (21°C, a late spring temperature, and 30°C, a high late summer temperature), and with and without partners. Thus at each temperature (21° and 30°) there were four treatments to which flatworms were haphazardly assigned: fed flatworms held singly or in size-matched pairs; unfed flatworms held singly or in pairs. Treatments at 21°C were made in spring and summer 1987. Treatments at 30°C were made in summer 1987 when field temperatures reached 29°C. All animals used in a 5-week period began a treatment at the same time and were observed every 48 h until they died or the 5-week period ended. A total of 103 single individuals and 45 pairs of flatworms survived the 5-week exposure. No animals were used more than once. Reproductive effort was measured as described earlier, except where noted in the results. It was not possible to ascertain if one or both animals in a pair had laid the eggs in any batch present in culture dishes after 48 h. Thus, for paired animals, we divided the number of eggs in any batch they produced by two to allow for a comparison with egg production by solitary animals.

Experiment B: Effects of temporal variations in food supply and absence of a partner

During spring and summer 1987, an attempt was made to determine how sensitive solitary flatworms were to long-term isolation and starvation, as evidenced by changes in

reproductive effort. Twenty-one flatworms were collected in April, measured, and held singly in the dark at 21°C. They were starved for 5 weeks to attempt to deplete any egg supply that might have been produced before capture (a 5-week period was used for symmetry with Experiment A). The water was changed every 48 h, but the logistical effort that went into initiating Experiment A prevented data collection during this initial 5-week period of Experiment B. The flatworms were then fed for 5 weeks, starved for 5 weeks, then fed again for 5 weeks to estimate their resilience to such changes. Data were collected and analyzed as in Experiment A. The four 5-week periods were designated First Starvation Period (only degrowth data collected), First Feeding Period, Second Starvation Period, and Second Feeding Period, respectively.

Results

Stylochus ellipticus laid eggs whether food or a partner were present or not. Egg diameter ranged from 61 to 74 μm ($\bar{x} \pm \text{S.E.} = 68.5 \pm 0.45 \mu\text{m}$; $n = 50$). Numbers of eggs within a batch (unadjusted for flatworm size) ranged from 125 to 85,600 for paired flatworms (these numbers were not divided by two) and from 295 to 39,300 for individuals. Up to 14 batches were produced (in the latter instance by a 93 mm^2 individual that also laid the most eggs—156,000 distributed over the 14 batches).

Mortality in Experiment A varied from 0 to 23% at 21°C and from 22 to 78% at 30°C, with no clear pattern of mortality associated with feeding state or presence or absence of a partner. Mortality was 19% at the end of 20 weeks at 21°C in Experiment B.

Size-related observations

Before we consider the main subject of the influence of temperature, food, and presence or absence of a partner on reproductive effort, we examine some findings that involve flatworm size. For Experiment A, our data are for solitary animals because it was not possible to distinguish between individual members of most pairs in order to follow their growth or degrowth.

Size changes in solitary flatworms. Starvation, accumulated exposure to stress, and high temperature had negative effects on flatworm growth.

At 21°C in Experiments A and B, most fed flatworms increased in area, whereas all unfed flatworms shrank

Figure 2. *Experiment B* Percentage increase (+) or decrease (–) in size (length \times width) of solitary *Stylochus ellipticus* at 21°C, under conditions of successive periods of starvation (First), feeding (First), starvation (Second), and feeding (Second), with each period being 5 weeks long. Note that the scale breaks across all four panels indicate transition between growth (+) and degrowth (–). Arrowheads on the y-axes of the starvation periods indicate average decrease in size. In the First Starvation panel, one animal decreased only 2% in size, and numeral (2) indicates where data for two other animals coincide.

(Figs. 1, 2). Fed animals that were smallest at the start of the experiments had the greatest increase in growth (up to about 1000% over their initial size); percentage change in growth declined with increasing initial size for fed animals. In terms of accumulated exposure to stressors, in Experiment B (Fig. 2), flatworms that survived the initial 5-week period of starvation grew more during the 5-week First Feeding Period (range of about 50–1000% increase over their initial size) than those in the Second Feeding Period (range of about 10–200% increase in size).

At 21°C in Experiments A and B, degrowth was relatively constant across the initial size range of unfed animals, with an average percentage change in size of –48% in Experiment A (Fig. 1) and –31% and –44% by the end of the First Starvation Period and Second Starvation Period of Experiment B, respectively (Fig. 2).

High temperature during Experiment A affected growth negatively, especially in concert with starvation. At 30°C, most fed individuals shrank (average decrease = –56%; Fig. 1, inset) in contrast to only four fed animals at 21°C (Fig. 1). As happened at 21°C, unfed flatworms at 30°C shrank at a relatively constant rate across the experimental size range, but degrowth occurred to a greater extent at 30°C (average decrease = –68%; Fig. 1, inset) than at 21°C (average decrease = –48%; Fig. 1).

Number of batches of eggs and flatworm size. Most flatworms smaller than 40 mm² produced no batches of eggs (Figs. 3, 4). There was a slight tendency for number of batches to increase with increasing area, but this was statistically significant only for the unfed animals at 30°C (a high stress condition) in Experiment A (Fig. 3).

Egg production and flatworm size. In Experiments A and B, especially for fed animals, the average area-specific number of eggs (*i.e.*, adjusted for flatworm area) produced per batch for solitary flatworms appeared to decline as initial area of the flatworms increased (Figs. 5, 6). However, this apparent decline was not statistically significant for any group.

Egg production and number of batches

Variability about the average area-specific number of eggs produced per batch was high in both experiments (Figs. 7, 8). Individual analysis of variance of the four sets of data in Experiment A (two temperatures; fed and unfed) and the three in Experiment B (First and Second Feeding, Second Starvation) revealed no significant differences in area-specific egg production per batch for any of the seven experimental conditions, undoubtedly because of this high variability in egg production.

Egg production and stressors

The overall (or grand mean) egg production in Experiments A and B was negatively affected by stress, with high

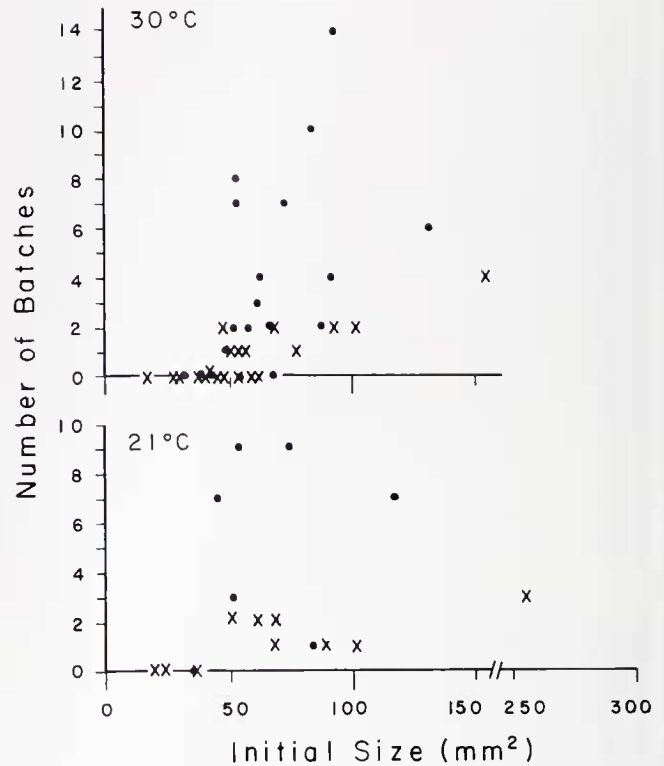


Figure 3. Experiment A. Number of batches of eggs in relation to initial size (length \times width) of fed (●) or unfed (>) solitary *S. ellipticus* after 5 weeks at 21° and 30°C.

temperature and lack of food generally depressing egg production in Experiment A, as did two starvation periods in Experiment B. In Experiment A, fed individuals produced an overall average of 150 eggs mm⁻² per batch at 21°C and 82 eggs mm⁻² per batch at 30°C (Fig. 7). In contrast, unfed individuals produced an overall average of 45 eggs mm⁻² per batch at 21°C and 55 eggs mm⁻² per batch at 30°C (Fig. 7). Experiment B flatworms produced an overall average of 163 eggs mm⁻² per batch during First Feeding (F¹, Fig. 8), a value similar to the 150 eggs mm⁻² per batch reported above for fed individuals at 21°C in Experiment A. As stress accumulated, Experiment B flatworms produced an overall average of only 52 eggs mm⁻² per batch during Second Feeding (F², Fig. 8), after having experienced two periods of starvation; they produced only 26 eggs mm⁻² per batch on average during the Second Starvation period (U, Fig. 8).

Experiment A: Effects of temperature, food, and presence or absence of a partner

Split-level ANOVA of our data grouped by four attributes (number of batches, days to first batch, percent

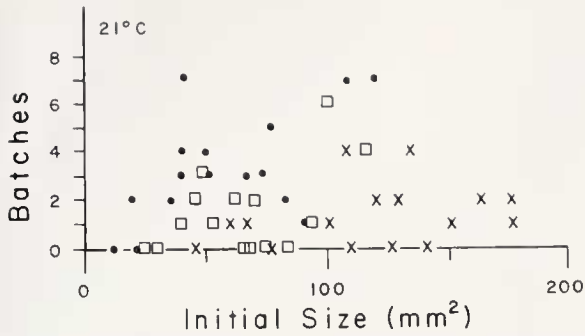


Figure 4. Experiment B Number of batches of eggs in relation to size (length \cdot width) of 16 solitary individuals of *Stylochus ellipticus* at 21°C. Animals had been starved for 5 weeks, then fed for 5 weeks (●), starved for 5 more weeks (×), then fed for 5 weeks (□). Initial size refers to size at the start of each of the 5-week periods. Only 15 square symbols are presented for second feeding flatworms because one animal's size was not recorded (it produced no eggs).

hatch, eggs per batch) produced complicated results (Table I). Because these experiments were made during different months (March–August), we performed a covariate analysis with day of the year to determine if the results were affected by time of year the experiment was performed. For two attributes (number of batches and percent hatch)

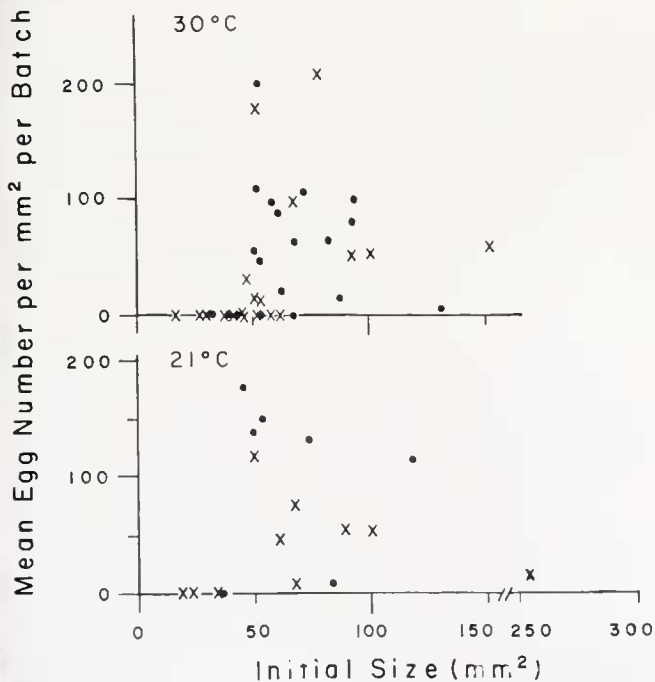


Figure 5. Experiment A Average number of eggs produced per batch by solitary individuals of *Stylochus ellipticus*, adjusted for initial flatworm size at the start of the experiment and plotted in relation to initial size. Data are for flatworms fed (●) or starved (×) for 5 weeks at 21°C or 30°C.

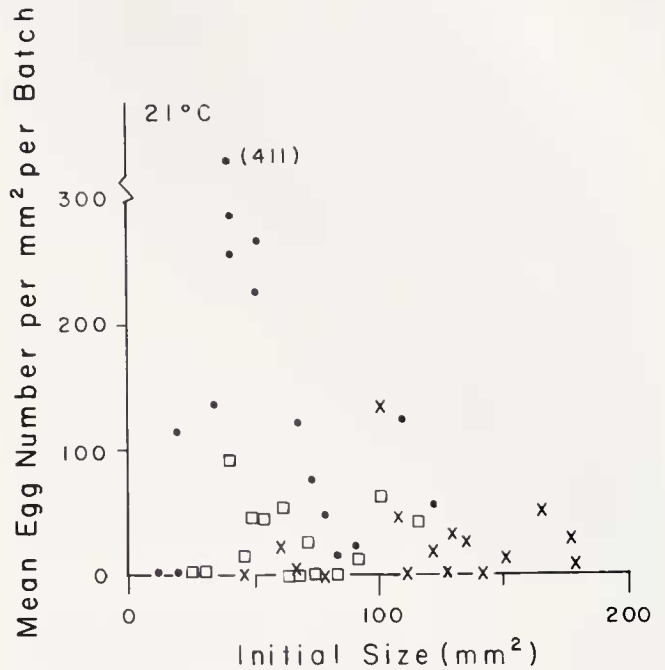


Figure 6. Experiment B Average number of eggs produced per batch by 16 solitary individuals of *Stylochus ellipticus*, adjusted for initial flatworm size at the start of the experiment and plotted in relation to size. Animals had been starved for 5 weeks, then fed for 5 weeks (●), starved for 5 more weeks (×), then fed for 5 weeks (□). Initial size refers to size at the start of each of the 5-week periods. Only 15 square symbols are presented for second feeding flatworms because one animal's size was not recorded (it produced no eggs).

there was a significant effect ($P \leq 0.1$) due to day of year (Table I). It is difficult to determine the reasons for the differences in number of batches because the four-way interaction of day of year \cdot status \cdot temp \cdot food was significant ($P \leq 0.1$) for that attribute (Table I). Day of year combined with factors like temperature and status showed a significant effect ($P \leq 0.1$) on percent hatch. An examination of the interactive effects on flatworm reproduction of temperature, feeding, and status, independent of day, showed they were insignificant, except for number of eggs per batch (Table I).

Temperature effects on flatworm reproduction were the most pronounced of the three manipulations. Values for the percent of flatworms (solitary or paired) laying eggs were generally higher at 21°C than at 30°C, but the average time to produce a first batch of eggs was significantly faster at 30°C than at 21°C (Tables II, III). The average number of batches produced was similar between the two temperatures for solitary animals, and fewer at 30°C when paired. There was a tendency for unfed solitary and paired animals at 30°C to lay more eggs per batch than at 21°C. Hatching success remained high (median values of 93–100%) under all conditions. In these comparisons for solitary and paired flatworms, statistically significant differ-

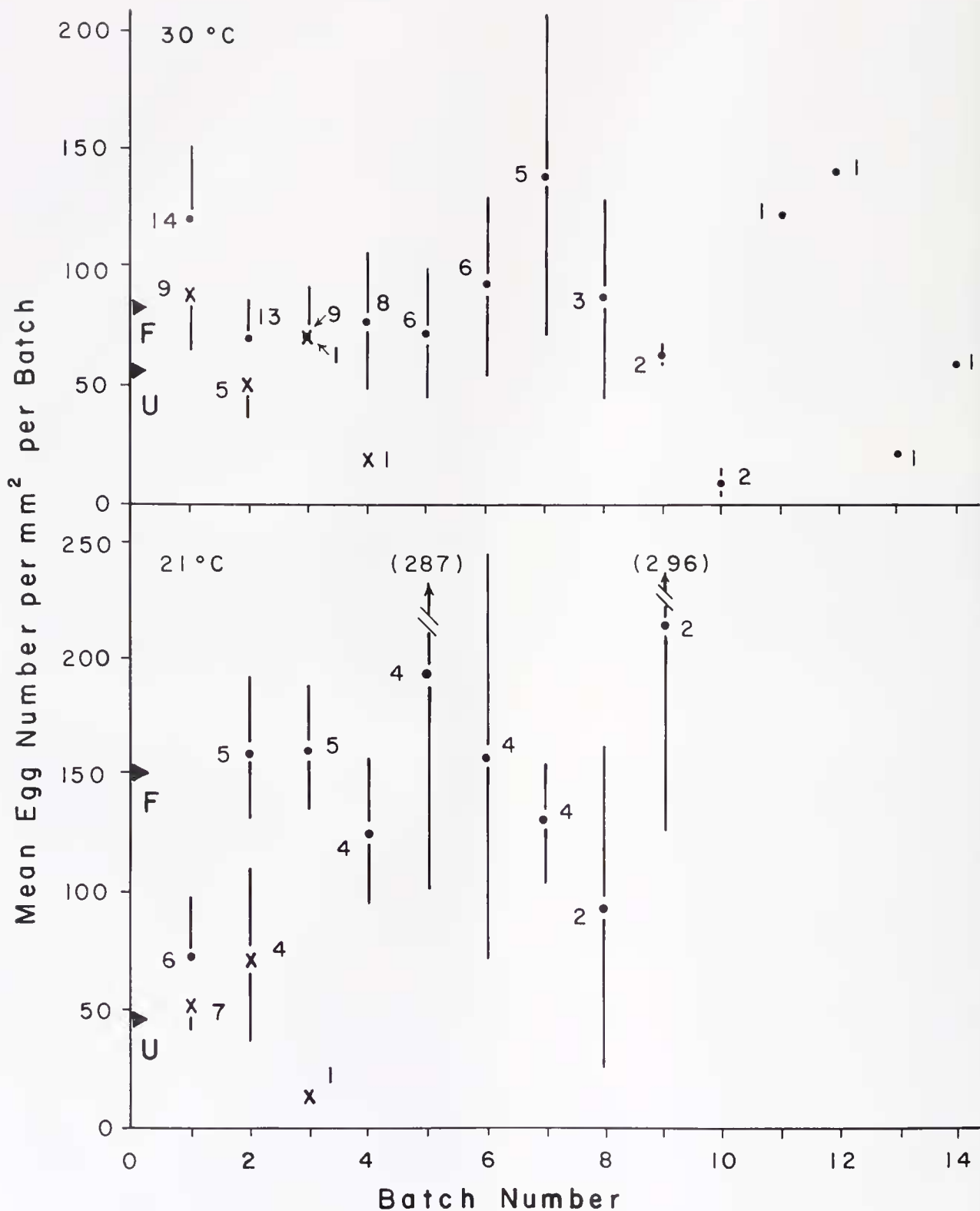


Figure 7. Experiment 1. Average number (± 1 SE) of eggs produced per batch by solitary individuals of *Stylochus ellipticus*, adjusted for flatworm size at the start of the experiment and plotted in relation to batch number. Data are for flatworms fed (\bullet) or starved (\cdot) for 5 weeks at 21°C or 30°C. Number of flatworms producing a batch are indicated. Arrowheads on the x-axis indicate grand means of eggs produced per batch under the experimental conditions of being fed (F) or unfed (U).

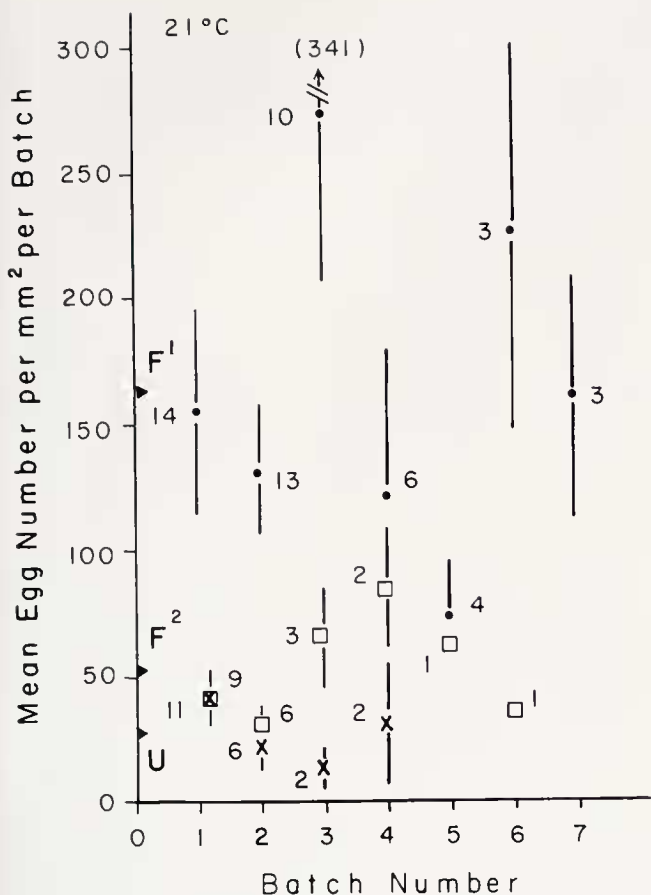


Figure 8. Experiment B. Average number (± 1 SE) of eggs produced per batch by 16 solitary individuals of *Stylochus ellipticus*, adjusted for flatworm size at the start of the experiment and plotted in relation to batch number. Number of flatworms producing a batch are indicated. Animals had been starved for 5 weeks, then fed for 5 weeks (●, first feeding), starved for 5 more weeks (×, second starvation), then fed for 5 weeks (□, second feeding). Arrowheads on the y-axis indicate grand means of eggs produced per batch under the experimental conditions of first feeding (F¹), second starvation (U), or second feeding (F²).

ences ($P \leq 0.1$) between temperatures occurred only in days to first batch (Table I).

We multiplied the average number of eggs per batch by the average number of batches produced, and multiplied the resultant estimate of total eggs laid by the median hatching percentage to derive an estimate of total larvae that would be produced in each treatment (Tables II, III). Except for unfed solitary flatworms, more larvae would result at 21°C than at 30°C.

Food availability also affected the reproductive activities of *S. ellipticus*. The proportion of animals that produced no batches was about twice as high for unfed animals (30% at 21°C; 53% at 30°C) than for fed animals (14% at 21°C; 26% at 30°C) (Fig. 3): note that the proportion producing no batches whether unfed or fed was about twice as high at 30°C as at 21°C. Lack of food had a

statistically significant effect ($P \leq 0.1$) on the onset of egg laying, with days to first batch generally declining with starvation (Tables II, III). We estimated that fed flatworms (paired or solitary) would produce more eggs than would unfed flatworms over the course of time, resulting in the production of more larvae despite a sometimes higher percentage of hatching for unfed flatworms (Tables II, III).

Solitary flatworms sometimes took longer average times than paired to produce their first batch of eggs (Tables II, III), but time differences over the range of experiments were not statistically significant for status (Table I). Average numbers of batches also were not significantly different between paired and solitary flatworms. Paired *S. ellipticus* individuals laid from 1.5 to 3.5 times fewer eggs per batch (recall that the number of eggs produced by a pair has been halved for this comparison) and consequently produced fewer larvae than solitary animals (Tables II, III); this difference was statistically significant ($P \leq 0.1$).

Experiment B: Effects of temporal variations in food supply and absence of a partner

When flatworms were held singly at 21°C for twenty weeks, 56% were still able to lay eggs when the experiment

Table I

Experiment A - Statistically significant results of manipulations based on split-level ANOVA

Attribute	Source	Type III SS	F-Value
Number of batches	Temp	27.25	4.53*
	Food	101.35	16.83**
	Day	31.68	5.26*
	Day*Status	49.59	10.47**
	Day*Food	29.75	6.28*
	Day*Temp*Food	13.03	2.75 ^a
	Day*Status*Temp*Food	22.47	4.74*
Days to first batch	Temp	13.46	29.10**
	Food	2.70	5.83*
Percent hatch	Status	1285.86	8.63**
	Temp	750.21	5.03*
	Food	2010.29	13.49**
	Day	765.91	5.14*
	Day*Status	1125.75	7.83**
	Day*Temp	1030.48	7.17**
	Day*Status*Temp	922.56	6.42*
Eggs/batch	Status	514462964.99	19.17**
	Food	86836259.36	3.24 ^a
	Status*Food	79686364.38	2.97 ^a
	Temp*Food	180828017.83	6.74*

"Type III SS" refers to the sum-of-squares value. In the F-value column, significant values are indicated as $P \leq 0.1$ (^a), $P \leq 0.05$ (*) or $P \leq 0.01$ (**). "Status" refers to presence or absence of a partner; "Food" to presence or absence of food; "Temp" to 21° or 30°C; "Day" to day of the year for experiments within a treatment. Degrees of freedom for all comparisons were 1.

Table II

Experiment A Data for nine separate sets of observations on reproductive effort for solitary *Stylochus ellipticus* that survived, fed and unfed at 21°C and 30°C, for a 5-week period

Attribute	Solitary									
	Fed				Unfed					
	21°C		30°C		21°C		30°C			
1. Sample size after 5 weeks	24	7	9	10	23	7	3	9	11	
2. Number laying eggs (%)	17 (71)	6 (86)	5 (56)	9 (90)	11 (52)	5 (71)	2 (67)	5 (56)	4 (36)	
3. \bar{X} Days to first batch	14.6 ± 2.3	10.3 ± 3.2	3.2 ± 0.8	2.9 ± 0.4	10.4 ± 2.5	8.2 ± 2.5	3.0 ± 1.0	2.8 ± 0.8	4.0 ± 0.8	
4. \bar{X} Number of batches	2.9 ± 0.5	6.0 ± 1.3	5.6 ± 1.5	4.9 ± 1.4	2.8 ± 0.5	1.6 ± 0.2	2.0 ± 1.0	2.2 ± 0.5	1.3 ± 0.3	
5. \bar{X} Eggs per batch	ND	9400 ± 1200	5300 ± 1280	7000 ± 1000	ND	4200 ± 920	4600 ± 1630	5500 ± 1380	7800 ± 2510	
6. Total eggs, thousands (row 4 × row 5)	ND	56	30	34	ND	7	9	12	10	
7. Percent hatch										
range	0-100	72-100	49-100	35-100	48-100	66-100	100	94-100	94-100	
median	97	99	97	99	99	98	100	98	98	
8. Total larvae, thousands (row 6 × row 7, median)	ND	55	29	34	ND	7	9	12	10	

Values are mean ± S.E. ND = eggs not counted.

ended, even after 20 weeks of alternate starvation and feeding (Table IV). In two instances, flatworms did not lay their first batch of eggs until after they were starved for the second time and then had been fed, *i.e.*, weeks 16-20 of the experiment.

In Experiment B, the percentage of flatworms laying eggs declined with time (Table IV). For attributes 3, 4, and 7, but not attribute 5, there was no significant difference between the First Feeding Period and the Second Feeding Period. Likewise, there was no significant difference between the Second Starvation Period and the Second Feeding Period for all four attributes except days to first batch (Table IV). The average number of batches produced per flatworm during the Second Starvation Period (1.9) was significantly lower than the numbers during the First Feeding Period (3.9) but not during the Second Feeding Period (2.4), in agreement with the decline in batch production with starvation in Experiment A (Tables II, III). Two flatworms in the First Feeding Period, five in the Second Starvation Period, and six in the Second Feeding Period produced no eggs (Fig. 4). The average number of eggs per batch decreased with time, so that the calculated total output of eggs decreased significantly ($P \leq 0.05$) from about 36,000 eggs during the First Feeding Period to about 9000 in the Second Feeding Period.

Over the three experimental periods, the median percentage of eggs that hatched into viable larvae remained

between 93 and 97%, with hatching success varying directly with feeding. As a result, as the total number of eggs changed with experimental conditions, the calculated total number of larvae also changed (Table IV).

The results of Experiment B (Table IV) can be compared with those for solitary flatworms at 21°C in Experiment A (Table II). At the end of the First Feeding Period in Experiment B, the percent laying eggs, the average number of days to first batch production, the average number of batches, the average number of eggs per batch, and the median percent hatching success (Table IV) were within the ranges of, or were similar to, values in Experiment A for the two sets of fed animals at 21°C (Table II). This was true also when results from the Second Starvation Period in Experiment B (Table IV) were compared with data for three sets of starved animals at 21°C in Experiment A (Table II), except for average hatching success which was lower in Experiment B. Values for attributes for the Second Feeding Period in Experiment B (Table IV) were lower (except for average number of days to first batch) than for fed flatworms at 21°C in Experiment A (Table II). Total egg and larval production at the end of 20 weeks was much less in Experiment B (Table IV) than in Experiment A after 5 weeks at 21°C (Table II). The overall similarities in attribute responses between fed animals in Experiment A and in Experiment B (First Feeding Period) and between starved animals from the two ex-

Table III

Experiment A Data for five separate sets of observations on reproductive effort for paired *Stylochus ellipticus* that survived, fed and unfed at 21°C and 30°C, for a 5-week period

Attribute	Paired				
	Fed		Unfed		
	21°C	30°C	21°C	30°C	
1. Sample size after 5 weeks	6	9	6	13	11
2. Number laying eggs (%)	5 (83)	6 (67)	5 (83)	12 (92)	7 (64)
3. \bar{X} Days to first batch	9.8 ± 2.7	4.7 ± 1.9	6.0 ± 2.5	5.3 ± 1.6	2.9 ± 0.9
4. \bar{X} Number of batches	6.8 ± 2.0	4.5 ± 1.0	2.8 ± 1.1	4.0 ± 0.6	1.7 ± 0.4
5. \bar{X} Eggs per batch ^a	4200 ± 610	2200 ± 400	2400 ± 760	3000 ± 600	3700 ± 1170
6. Total eggs, thousands (row 4 × row 5)	29	10	7	12	6
7. Percent hatch range	62–100	26–100	93–100	65–100	65–100
median	97	93	99	98	98
8. Total larvae, thousands (row 6 × row 7, median)	28	9	7	12	6

Values are mean ± S.E.

^a Adjusted by dividing by two to allow comparison with solitary flatworms.

periments indicate that the patterns uncovered in the two experiments are indeed repeatable and not specific to a single experiment.

Summary of results

Our experiments produced complex results, so we summarize the significant findings here. Higher temperature and starvation negatively affected flatworm growth (Figs. 1, 2). When fed, smaller flatworms grew proportionately more than did larger flatworms; all unfed animals tended to shrink to a proportionately similar extent, with higher temperature and accumulated exposure to starvation associated with greater degrowth (Figs. 1, 2). The number of batches of eggs produced (Figs. 3, 4) and (when egg production was adjusted for area of solitary flatworms) the average number of eggs produced per batch (Figs. 5, 6) were not significantly affected by initial area of the flatworms. Similarly, the average number of eggs produced per batch, adjusted for flatworm area, did not change significantly as more batches were produced (Figs. 7, 8).

There was no significant statistical effect of higher temperature and starvation on egg production, although there were some instances of diminished egg production under these conditions (Figs. 7, 8; Tables II–IV). Flatworms did react to higher temperature and starvation by significantly decreasing the number of days that elapsed before egg laying began (Tables II–IV). Overall, more larvae would be produced at 21°C than at 30°C (Tables II, III), by fed flatworms than by starved flatworms (Tables II–IV), and

by solitary flatworms than by paired flatworms (Tables II, III).

Discussion

Flatworms in our experiments increased or decreased in surface area relative to the availability of food. Pearse and Wharton (1938) found in Florida that fed *S. ellipticus* (which they called *Eustylochus meridionalis*) could grow from an average of 1.4 to 5.2 mm (n = 5) in 17 days in March, and that starved *Stylochus frontalis* experienced an average 30% decrease in size from November 18 to March 5, a slightly lower percentage than we found. In our study, smaller, fed animals grew the most proportionally, while larger flatworms grew the least. In Experiment B, the lessened growth in the Second Feeding Period compared with growth in the First Feeding Period (Fig. 2) may have resulted from the accumulated stress of two periods of starvation, or from increasing age of the flatworms; the lack of continuously fed controls hinders a conclusion.

Flatworms that were held for a long period under stress (lack of food, high temperature) could produce many eggs while at the same time undergoing degrowth (Tables II–IV). This may be an indication that the flatworms diverted resources from growth and maintenance to reproduction, resulting in a decreased body size. A similar strategy has been reported in mollusks (Havenhand and Todd, 1989). If the cost of reproduction results in an inability of the reproducing adult to meet all its energy requirements from

Table IV

Experiment B Data for reproductive effort of solitary *Stylochus ellipticus* held at 21°C for 20 weeks under alternating feeding and starvation regimes of 5 weeks each

Attribute	First feeding	Second starvation	Second feeding
1. Sample size after each 5-week period	21	20	16
2. Number laying eggs (%)	14 (67)	11 (55)	9 (56)
3. \bar{X} Days to first batch	12.4 ± 2.04 ^z	6.2 ± 1.69	13.0 ± 3.55 ^z
4. \bar{X} Number of batches	3.9 ± 0.53 ^z	1.9 ± 0.34 ^y	2.4 ± 0.56 ^{yz}
5. \bar{X} Eggs per batch	9200 ± 1100	4000 ± 800 ^z	3700 ± 650 ^z
6. Total eggs, thousands (row 4 × row 5)	36	8	9
7. Percent hatch range	5–100	12–100	29–100
median	97 ^z	93 ^y	94 ^{yz}
8. Total larvae, thousands (row 6 × row 7, median)	35	7	8

Values are means ± S.E. Values with like superscripts for attributes 3, 4, 5, and 7 are not significantly different at $P \leq 0.05$.

input energy, the deficit may be made good in *S. ellipticus*, as in trielads, from the catabolism of tissue or degrowth (Woollhead and Calow, 1979). This capacity for degrowth may be especially important during the reproductive period if food supply is reduced through inter- and intra-specific competition for food (Woollhead, 1983).

Life history features such as how much resource to put into reproduction must be the outcome of compromises between conflicting demands (Grahame and Branch, 1985). The most prominent life-history compromise involves the cost of reproduction, with costs paid in survival or in future reproduction (Stearns, 1989). For *S. ellipticus*, the reproductive effort of the flatworm is maintained while the flatworm decreases in size, rather than ceasing at the same time that size is being maintained.

Stylochus ellipticus was able to produce numerous fertile eggs whether solitary or paired, fed or unfed, and at 21° or 30°C. In this it appears to differ from many other turbellarians that are reported by Henley (1974) to show rapid regressive changes in their reproductive systems under adverse environmental conditions.

Stylochus ellipticus in Chesapeake Bay has the potential to be reproductively active in spring, summer, and early autumn, based on the fact that it was able to produce eggs at both 21°C and 30°C. Flatworms produced eggs in our study from March through August (we did not examine

them in other months). In Florida, Pearse and Wharton (1938) found *S. ellipticus* to continue to lay eggs when brought into the laboratory in winter and spring, but there appears to be no information on the effects of the lower temperatures of the northern winter on gametogenesis and reproduction.

The fact that reproductive output of *S. ellipticus* (as measured by total eggs and larvae produced) was usually greater for animals at 21°C than 30°C in our study suggests that the optimal period for reproduction in nature is most likely spring or autumn when field temperatures are lower than in summer. Dumont and Schorreels (1990) likewise found 20°C to be the optimum temperature for reproduction in the turbellarian *Mesostoma lingua*.

There are limited spawning data available for other species of *Stylochus*. Two species show evidence of prolonged spawning for six months or more, i.e., *S. mediterraneus* in Italy (Galleni *et al.*, 1980) and *S. inimicus* (?) in Taiwan (Kuo *et al.*, 1984). This is also true for other polyclads, including *Pseudostylochus ostreophagus* (Woelke, 1957) and *Notoplana acticola* (Thum, 1974; see also Kato, 1940). However, *S. zebra* only produces eggs from June through August (Lytwyn and McDermott, 1976).

We have no data on how long *S. ellipticus* can survive in nature, but we collected individuals all year round in the Choptank River, Maryland (unpub. data), as did Pearse and Wharton (1938) at some stations in Florida. Large flatworms (>200 mm²) were not collected in the Choptank River from about November to March (Chintala, 1987), so it is possible that *S. ellipticus* is an annual species. *S. zebra* can live in the laboratory for over one year, although there is a lower abundance in nature after the summer reproductive period (Lytwyn and McDermott, 1976). Galleni *et al.* (1980) found *S. mediterraneus* in mussel beds year round, although the flatworms were rare in winter and spring. On the other hand, *Pseudostylochus ostreophagus* in Washington died in nature and in the laboratory during the midsummer of three successive years (Woelke, 1957).

Even after twenty weeks of isolation, 56% of the flatworms in Experiment B were still able to produce viable eggs. Thus, even though these animals may be non-self-fertilizing (Hyman, 1940, 1951; Prudhoe, 1985), and are thought to be internally fertilized (Pearse and Wharton, 1938; Giese and Kanatani, 1987), they were still reproductively active. If they do not self-fertilize, then either there must be some mechanism of sperm storage or else these animals could produce eggs capable of parthenogenic development when necessary in adverse conditions, such as when mates are absent. Self-fertilization is thought to be uncommon in Turbellaria because the male and female reproductive systems are largely separate, although a few trielads are parthenogenic (Gosner, 1971). It is possible

that sperm retention is the alternative mode used by these flatworms, because *S. ellipticus* has a seminal receptacle in which sperm could be stored (Prudhoe, 1985). *S. ellipticus* could be like the Pacific Coast species *Notoplana acticola* in which sperm is present in the seminal receptacle throughout the year, suggesting either that copulation occurs throughout the year or that sperm are readily stored for extended periods of time (Thum, 1974).

The eggs of *S. ellipticus* and some other species of *Stylochus* are relatively small in comparison with those of *S. zebra* and other polyclads (Table V). This small size is accompanied in *S. ellipticus* by the shortest development period before hatching reported for stylochids, and by the potential to produce tens of thousands of eggs per batch, with numerous batches possible. The small egg size and high fecundity, coupled with the short developmental period and extended breeding period, indicate that *S. ellipticus* is well adapted to resisting environmental stressors encountered in its estuarine habitat.

There was a distinct pattern in the flatworms' egg laying response to potential stressors (*i.e.*, high temperature, no food) and the condition of isolation. Earlier egg laying was significantly associated with increased temperature or decreased food, which could result in the animals contributing to the gene pool before they could possibly succumb to the effects of the high summer temperatures or, especially, to starvation. Perhaps this is because there is little point in withholding energy from reproduction if the adult is not likely to survive long enough to reproduce

at a later stage (Grahame and Branch, 1985). In addition, even though the flatworms responded to stress by de-growth, they may not have decreased the amount of material needed to maintain egg viability as reflected by the high hatching success of eggs over most stress conditions. For example, the percent of eggs hatching for solitary unfed individuals at 30°C was about as high as or higher than for unfed animals at 21°C and similar for fed animals at both temperatures (Table II). All these events suggest that when adult *S. ellipticus* are exposed to stress, they at least maintain the effort of producing young. Nevertheless, a variety of factors may determine when reproduction occurs, and at times these factors may conflict so that no single general cue for reproduction emerges (Grahame and Branch, 1985).

The concept of compensation means that if allocation to one function increases, then allocations to others will decrease (Grahame and Branch, 1985). Whether physiological compensations exist depends on the organism and what sort of breeding cycle it has. Two extremes on a continuum are "income" and "capital" breeders (Stearns, 1989). An income breeder uses its current income for reproductive investment, whereas a capital breeder stores energy that can be mobilized later for reproduction (Stearns, 1989). *S. ellipticus* resembles a capital breeder in that it can produce eggs while unfed, relying on stored energy.

Solitary flatworms sometimes took longer average times than paired flatworms to produce eggs, but the data were

Table V

Size and number of eggs laid in a batch, and length of time before hatching occurs for polyclad Turbellaria

Species	Egg number	Egg size (µm)	Development period (days)	T (°C)	Reference
<i>Stylochus ellipticus</i>	63-42,800 (pair) 295-39,300 (individual)	61-74	5-7	21	This study
<i>S. aomori</i>	—	95-105	8	"early summer"	Kato (1940)
<i>S. frontalis</i>	919-18,028	—	11	28	Pearse and Wharton (1938)
(= <i>immicus</i>)	^a	—	10-17	26	Kuo <i>et al.</i> (1984)
<i>S. mediterraneus</i>	—	—	8	22	Bytinski-Salz (1935)
			8-10		Galleni <i>et al.</i> (1980)
<i>S. uniporis</i>	—	85-95	8	"early summer"	Kato (1940)
<i>S. zebra</i>	220-487 ^b	200-220	14	25.5	Lytwyn and McDermott (1976)
			17	22	
<i>Euplana gracilis</i>	25-400	85-100	7	21	Christensen (1971)
<i>Notoplana australis</i>	"several hundred"	120-125	12-14	20-22	Anderson (1977)
<i>Pseudostylochus ostreophagus</i>	3373-88,332 ^c	147	30-34	15-17	Woelke (1957)

T—incubation temperature. RT = room temperature (not stated). Egg numbers reported for *Stylochus ellipticus* pairs in this study have been divided by two.

^a Largest produced 450,000 eggs in seven batches; smallest produced 93,000 in four batches.

^b Maximum number over 23 days was 9549 eggs.

^c Number of eggs laid in a season (March-October).

not consistent enough to suggest that the presence of a partner stimulates egg laying. The number of eggs laid per flatworm was significantly higher for solitary than for paired animals; however, the number of eggs per batch was not significantly different. This suggests that the solitary animals might decrease size more and put the same amount of effort into reproduction as do animals that are paired. Another possibility is that when animals are paired, both reduce their allocation of resources to reproduction or only one animal does the egg laying. The fact that a solitary individual produces significantly more eggs is intriguing. If, when populations are low in nature, the lack of stimulation by other flatworms causes solitary individuals to lay more eggs, then the population could grow. The presence of a partner would not lead to a concomitant increase in fecundity, thus stabilizing the population.

Although *S. ellipticus* reacted to perturbations in food supply by growing or shrinking, it continued to put much effort into reproduction. In this it resembles semelparous species that put more effort into reproduction than do iteroparous species (Calow and Woollhead, 1977; Woollhead and Calow, 1979; Woollhead, 1983). Semelparous species also are less sensitive to perturbations in food supply and continue their reproductive output despite reductions in food supply. Or, *S. ellipticus* could be like the gastropod *Nassarius pauperatus*, which produces more eggs when food is short, thus shifting from iteroparity to semelparity when future adult survival is threatened (McKillup and Butler, 1979). However, our data are only preliminary and more work is required to answer the question about semelparity in *S. ellipticus*.

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